A MICROFLUIDIC DEVICE FOR LONGITUDINAL STUDIES OF *C.ELEGANS* NEURODEGENERATIVE DISEASE MODELS

M. Cornaglia*, G. Krishnamani, L. Mouchiroud, T. Lehnert, J. Auwerx, and M.A.M. Gijs
Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, SWITZERLAND

ABSTRACT

We describe a microfluidic device for automated culture and long-term high-resolution imaging of *Caenorhabditis elegans* nematodes, which we specifically employed as model organisms for the analysis of neurodegenerative disease progression. In this platform, we integrated: (i) a microfluidic design tailored for the confinement of worms at the L2 larval stage in separate culture chambers by means of passive hydrodynamics; (ii) an optimized protocol for worm feeding and progeny removal at desired rate, allowing follow-up of the same worms over long-term studies; (iii) a technique for reversible *C.elegans* immobilization, based on the thermoreversible gelation of Pluronic F127 (PF127) inside the microfluidic chip; (iv) active control of the device temperature; (v) a compact device assembly, suitable for automated multi-dimensional imaging on any upright or inverted microscope.

KEYWORDS: *C.elegans*, neurodegenerative disease, amyotrophic lateral sclerosis (ALS), protein aggregation, longitudinal time-resolved analysis, high-resolution imaging, worm immobilization, temperature control, microfluidics

INTRODUCTION

C.elegans represents one of the best model organisms for long-term longitudinal analyses, mainly because of its fast life cycle, combined with the ease of its genetic manipulation. Complex biological processes related to human dysfunctions developing throughout many years – e.g. neurodegenerative diseases – could be investigated in *C.elegans* in a matter of a few days or even hours [1]. Because of its small size (hundreds of microns), however, high-magnification imaging is usually needed to extract relevant biological information, while accurate tools for worm manipulation are required to preserve the identity of individual worms over longitudinal studies [2]. We recently introduced a platform for automated worm culture and validated its potential for long-term high-resolution imaging of small *C.elegans* populations [3]. We now further extend this concept by providing our platform with a new microfluidic design and employ it for longitudinal studies at single-worm resolution.

RESULTS AND DISCUSSION

Our microfluidic device exploits the thermoreversible gelation of the triblock copolymer PF127 for reversible worm immobilization [4], using active temperature control (Figure 1a,b), both to set precise environmental conditions for *C.elegans* maintenance and to steer the worm immobilization/release process. In particular, in our system, a PDMS/glass chip is inserted in a metallic "thermalization frame", which is thermally connected to a Peltier module and insulated from the external environment by a custom-made microscope holder. A hole in the thermoelectric module ensures efficient light exposure of the chip for high-resolution transmission microscopy. The whole device sizes 60 mm in diameter, as standard petri dishes; the use of lateral fluidic connections enables device operation with both upright and inverted microscopes. Our new microfluidic design is tailored for the isolation of L2 larvae in separate chambers and for their automated culture and imaging over several days (Figure 1c,d). Worms are cultured on-chip at a desired temperature (typically 20°C) and fed by perfusing an *E.coli* suspension. Prior to a high-resolution imaging session, the microfluidic chip is cooled down to 15°C to allow the injection of PF127 solution (25% w/v in water) in liquid phase. The chip temperature is then raised to 25°C to trigger the gelation of the PF127 solution for worm immobilization. Closed-loop regulation of the device temperature is handled via a proportional-integral-derivative (PID)-controlled Peltier module, in contact with the chip. We optimized its performance to ensure fast and reliable temperature setpoints

with minimal fluctuations beyond the preferred temperature range for *C.elegans culture* (15-25°C), over the whole microfluidic chip area (Figure 1e-f).

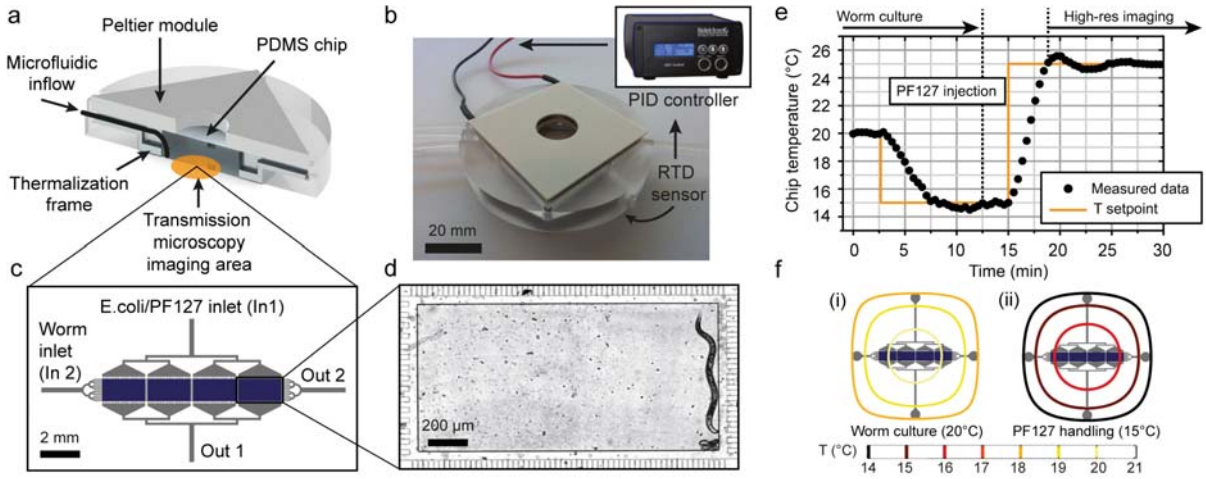

Figure 1: (a) Schematic representation (section view) and (b) photograph of the microfluidic device. (c) Schematic view of the microfluidic chip design, featuring 2 inlet/outlet sets along perpendicular directions and 4 worm culture chambers, interconnected by filtering channels. (d) Micrograph of a worm culture chamber hosting a young adult C.elegans nematode. (e) Experimental temperature at the chip center during a worm culture-to-imaging transition, as managed by the active temperature control system. (f) FEM simulation of the spatial temperature distribution over the microchip area, during (i) worm culture at 20°C and (ii) PF127 handling at 15°C. In both cases, temperature uniformity within 1°C difference is achieved over the whole chamber matrix.

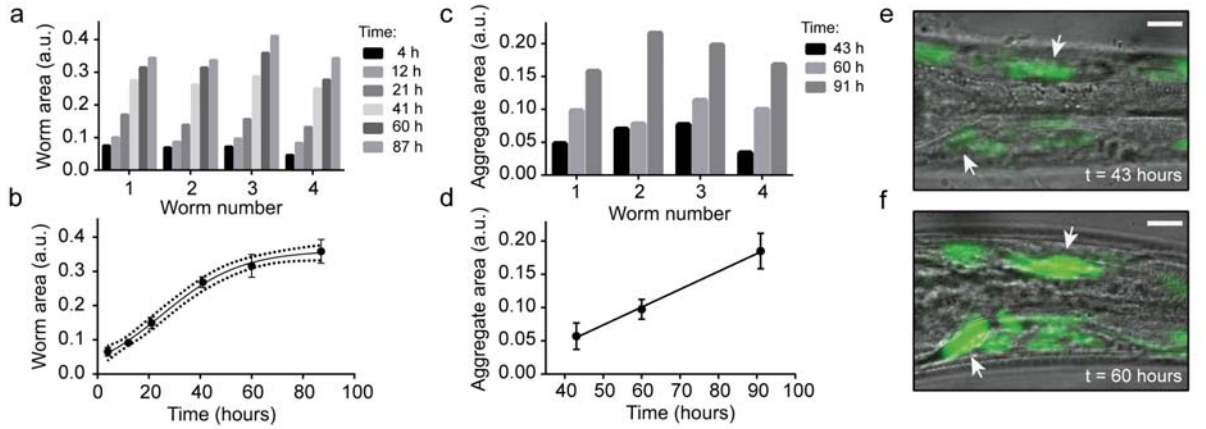

Figure 2: (a) On-chip growth rate of individual worms over 87 hours, as estimated by measuring the worm area over time-lapse pictures. (b) Average on-chip worm growth, featuring a sigmoidal time-dependence. (c) Growth rate of SOD1-YFP aggregates in the body wall muscle cells of each worm, as estimated by measuring YFP expression area across each worm's body during their immobilization. (d) Average protein aggregate area per worm, following a linear time-dependence over the period from 43 to 91 hours upon loading on chip (day 1 to day 3 of worm adulthood). (e-f) Representative high-resolution images of an immobilized worm, as taken through a 63x NA 1.4 oil immersion objective, (a) 43 hours and (b) 60 hours after worm loading into the device. Each brightfield picture is superimposed to the corresponding fluorescent image, to map the distribution of SOD1-YFP aggregates within the worm tissues at precise locations of its body. Reversible immobilization/imaging cycles allow moreover following the progression of specific protein aggregates over time (see arrows). Scale bars = 20 µm.

We successfully employed our device to monitor protein aggregation in a *C.elegans* model for amyotrophic lateral sclerosis (ALS), expressing mutated human SOD1-YFP fusion proteins in the body wall muscle cells (AM725 mutant) [5]. Via simple image-processing on time-lapse brightfield pictures, we automatically monitored the growth rate of individual worms within the microfluidic chambers over 4 days (Figure 2a-b). Similarly, by combining reversible worm immobilization with fluorescent imaging, we followed the progression of SOD1-YFP aggregates over time for each worm (Figure 2c-d). Moreover, by combining brightfield and fluorescent high-resolution images, we could precisely localize protein aggregates within the worms' tissues, as well as monitor the evolution of single aggregates over consecutive days (Figure 2e-f).

CONCLUSION

We introduced a new device for longitudinal studies on *C.elegans* nematodes down to single-worm resolution. We integrated different functionalities into a single miniaturized device, specifically designed for fully automated worm analyses. Active temperature control, together with robust on-chip protocols, allow in fact easy and reliable *C.elegans* culture and analysis in our microfluidic chips, without need of any calibration and no influence of external temperature or other environmental and human factors. On our platform we implemented on-chip reversible immobilization and long-term high-resolution imaging of *C.elegans* nematodes. We then demonstrated accurate monitoring of protein aggregation in worms' tissues over four consecutive days. We anticipate that our platform will be employed to address different biological questions related to protein aggregation and neurodegeneration in *C.elegans* models. Our device format will be moreover particularly suitable for drug tests, thus we envision wide application of our microfluidic technology for drug discovery purposes, especially in the neurodegenerative disease field.

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CONTACT

*M. Cornaglia, tel: +41 21 693 48 34; matteo.cornaglia@epfl.ch